which are substantially complementary to unique nucleic acid segments within the chromosomal DNA for which detection is desired, wherein the nucleic acid probe is substantially free of repetitive segments which are complementary to repetitive segments in the target interphase chromosomal material; and

(b) employing said labeled nucleic acid probe and chromosomal DNA in in situ hybridization so that hybridization of unique segments within the labeled nucleic acid probe to the chromosomal DNA is allowed, and hybridized labeled nucleic acid containing unique segments are detected, and wherein the interphase chromosomal DNA is present in a morphologically identifiable [chromosome or] cell nucleus during the in situ hybridization.

96. (Amended) The method of claim 72, wherein fragments substantially complementary to repetitive segments in the target interphase chromosomal material [have been] are removed from the labeled nucleic acid probe.

Please add the following new claims:

-- 98. A method of staining target chromosomal material comprising:

(a) providing at least one labeled nucleic acid probe having a complexity greater than about 50 kb, which labeled nucleic acid probe comprises fragments which are substantially complementary to unique nucleic acid segments within the chromosomal material for which detection is desired, and providing blocking nucleic acid that comprises fragments which are substantially complementary to repetitive segments in the labeled nucleic acid; and

(b) employing said labeled nucleic acid probe, blocking nucleic acid, and chromosomal DNA in *in situ* hybridization so that labeled repetitive segments, if present, are substantially blocked from binding to the chromosomal DNA, while hybridization of unique segments within the labeled nucleic acid probe to the chromosomal DNA is allowed, wherein blocking of the labeled repetitive segments is sufficient to permit detection of hybridized labeled nucleic acid containing unique segments, and wherein the chromosomal DNA is present in a morphologically identifiable chromosome or cell nucleus during the *in situ* hybridization.

99. The method of claim 98, wherein the target chromosomal material is present in an interphase cell nucleus.

100. The method of claim 99, wherein the labeled nucleic acid has a complexity of between about 50 kb and 400 kb.

101. The method of claim 100, wherein the labeled nucleic acid has a complexity between about 50 kb and 100 kb.

102. A method of staining target interphase chromosomal DNA comprising:

(a) providing at least one labeled nucleic acid probe having a complexity greater than about 50 kb which labeled nucleic acid probe comprises fragments which are substantially complementary to unique nucleic acid segments within the chromosomal DNA for which detection is desired, wherein the nucleic acid probe is

substantially free of repetitive segments which are complementary to repetitive segments in the target interphase chromosomal material; and

(b) employing said labeled nucleic acid probe and chromosomal DNA in *in situ* hybridization so that hybridization of unique segments within the labeled nucleic acid probe to the chromosomal DNA is allowed, and hybridized labeled nucleic acid containing unique segments are detected, and wherein the interphase chromosomal DNA is present in a morphologically identifiable cell nucleus during the *in situ* hybridization.

103. The method of claim 102, wherein the labeled nucleic acid has a complexity of between about 50 kb and 400 kb.

104. The method of claim 103, wherein the labeled nucleic acid has a complexity between about 50 kb and 100 kb.--

**REMARKS**

Entry of the foregoing and further and favorable reconsideration of the subject application pursuant to and consistent with 37 C.F.R. §1.112 is respectfully requested.

By the present amendment, the specification has been amended to correct the continuing application data in this case, as requested by the Examiner. New claims 98-104 have been added. These claims are directed to the same subject matter as claims 48, 62, 63, 72, 88, and 89, but recite that the probe complexity is

"about 50 kb," rather than "about 40 kb." These claims derive support from throughout the specification and claims as originally filed. No new matter has been added.

Turning now to the Official Action, claims 48-63, 65-69, 71-86, 88, 90-93, 95, and 96 are rejected under 35 U.S.C. §112, first paragraph, as purportedly unsupported by the specification as originally filed. This rejection, to the extent that it applies to the claims as amended, is respectfully traversed.

The Examiner asserts that the recitation in the present claims of probes with at least 40 kb of complexity introduces new matter into the claims. In particular, the Examiner argues, at pages 3-4 of the Official Action, that "all complexities for probes are described as being on the order of 50 kb or greater whereas the 40 kb size is only disclosed regarding a probe and not its complexity. " Applicants respectfully disagree, and maintain that the present specification provides ample explicit support for probes with a complexity of at least 40 kb. At page 13, lines 6-7 of the specification, Applicants note that "prior to this invention, probes employed for *in situ* hybridization techniques had complexities below **40 kb**, and more typically on the order of a few kb." At page 37, lines 24-26, Applicants point out that "from **about a 40 kb** to about a 100 kb target sequence may be presently necessary to provide a reliable, easily detectable signal." At page 38, lines 5-13, Applicants reiterate that,

> The term "complexity" therefore refers to the complexity of the total probe no matter how many visually distinct loci are to be detected, that is, regardless of the distribution of the target sites over the genome.
>
> As indicated above, with current hybridization techniques it is possible to obtain a reliable, easily detectable signal with a probe of **about 40 kb** to about 100 kb (eg. the probe insert capacity of one or a

> few cosmids) targeted to a compact point on the genome. Thus, for example, a complexity in the range of approximately 100 kb now permits hybridization to both sides of a tumor-specific translocation.

Further support may be found in the discussion of the use of mixtures of low complexity probes at page 40, lines 12-22:

> One method of forming the probes of the present invention is to pool many different low complexity probes. Such a probe would then comprise a "heterogenous mixture" of individual clones sequences. The number of clones required depends on the extent of the target area and the capacity of the cloning vector. If the target is made up of several discrete, compact loci, that is, single spots at the limit of microscopic resolution, then **about 40 kb**, more preferably 100 kb, for each spot gives a reliable signal given current techniques. The portion of the probe for each spot may be made up from, for example, a single insert from a yeast artificial chromosome (YAC), from several cosmids each containing 35-**40 kb** of probe sequence, or from about 25 plasmids each with 4 kb of sequence.

These citations from the instant application all cite the numerical value of 40 kb of complexity. In view of the foregoing, Applicants maintain that the pending claims fully comply with the requirement of 35 U.S.C. §112. Withdrawal of this rejection is thus respectfully requested.

Claims 72-74, 76-87, 88-93, and 95-97 are rejected under 35 U.S.C. §112, second paragraph, as purportedly indefinite. This rejection, to the extent that it applies to the claims as amended, is respectfully traversd.

The Examiner notes, at pages 4-5 of the Official Action, that the recitation of interphase chromosomes in certain claims appears to be incompatible with the simultaneous recitation of morphologically identifiable chromosomes. Without conceding to the Examiner's arguments, but solely in an effort to expedite

prosecution, claim 72 has been amended to delete the recitation of "morphologically identifiable chromosome."

The Examiner asserts that the recitation in claim 96 that the repetitive segments "have been" removed renders claim 96 indefinite. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, claim 96 has been amended to recite that the repetitive segments "are" removed.

In view of these amendments, withdrawal of this rejection is respectfully requested.

Claims 50 and 75 are rejected under 35 U.S.C. §112, fourth paragraph, as purportedly in improper dependent form. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, claims 50 and 75 have been deleted without prejudice or disclaimer, thus rendering this rejection moot.

Claims 72, 74-86, 88-93, and 95-97 are rejected under 35 U.S.C. §103 as purportedly obvious over U.S. Patent 4,710,465 to Weissman et al. in view of Lichter et al. *PNAS* 85:9664-9668, 1988). This rejection, to the extent that it applies to the claims as amended, is respectfully traversed.

The requirements of a *prima facie* case of obviousness are set forth in MPEP 2143:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The Examiner asserts, at page 7 of the Official Action, that Weissman et al. disclose

> in columns 5-6, bridging paragraph, that the invention therein described detects chromosomal arrangements such as the spacing between genes including linkage that may be related to disease. Probed regions are disclosed as 50 kb to 200 kb in column 9, lines 14-32, which clearly qualifies as high complexity probes as instantly claimed.

However, Applicants respectfully maintain that Weissman et al. does not discuss probe complexity at all. The passage pointed to by the Examiner refers to the length of probed (*i.e.,* target) regions to be stained, not to probe complexity.

Lichter et al. does not remedy this deficiency of Weissman et al. First, Lichter et al. begin with a probe set containing 94 kb of insert DNA (p. 9664, last paragraph of col. 2). Lichter et al. do not explicitly define this measurement as either length or complexity. However, Lichter et al. go on to state that "the probe concentration was decreased in proportion to the sequence complexity of the probe mixture." (sentence bridging pp 9664-9665), and conclude by pointing out that "when probe sets containing 29 kb or less of target sequence were used, the fluorescein isothiocyanate detection was generally enhanced by one cycle of signal amplification." (p. 9664, third paragraph in col 1). Thus, while Lichter et al does not explicitly address the complexity of their probe set, even if the 94 kb and 29 kb are assumed to refer to complexity, Lichter's assertion that use of 29 kb is advantageous teaches away from the use of probes of about 40 kb of complexity, about 50 kb, or greater, as recited in the instant claims.

> When prior art references require a selective combination to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself.

> Something in the prior art as a whole must suggest the desirability, and thus the obviousness of making the combination.

*Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 5 USPQ2d 1434 (Fed. Cir. 1988). Not only are all of the limitations of the present claims neither taught nor suggested by the cited publications, but also the cited art does not suggest the desirability of modifying the disclosures thereof in order to arrive at the present invention. Accordingly, the presently claimed invention is not *prima facie* obvious over Weissman et al. in view of Lichter et al. Withdrawal of this rejection is thus respectfully requested.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly obvious over claim 125 of copending application Serial No. 08/473,327. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly obvious over claims 1, 48, and 50-58 of copending application Serial No. 08/477,316. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly

obvious over claims 1 and 48-50 of copending application Serial No. 08/487,387. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly obvious over claims 62-65 and 125-148 of copending application Serial No. 08/478,740. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly obvious over claims 18-33 of copending application Serial No. 08/472,312. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

Claims 72, 74-86, 88-93, and 95-97 are provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as purportedly obvious over claims 131, 132, 144-147, and 150-153 of copending application Serial No. 08/487, 974. Without conceding to the Examiner's arguments, but solely in an effort to expedite prosecution, Applicants hereby express their willingness to file a

Terminal Disclaimer in these cases upon indication that the claims in both cases are otherwise allowable.

From the foregoing, further and favorable reconsideration in the form of a Notice of Allowance is believed to be next in order and such action is earnestly solicited.

In the event that there are any questions concerning this amendment, or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

Respectfully submitted,

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